effect over to pro-ABE I suggesting an influence on development of inter-exosite communication. As F1.2 is removed and PT2 is activated to thrombin, pro-ABE I and II become more solvent exposed and mature into the functional exosites ABE I and II. HSQC NMR titrations with GpIbalpha (269-286, 15N-labeled L275, 15N-labeled D277) demonstrate that ligand binding affinity at pro-ABE II / ABE II increases as ProT is converted to PT2 and then thrombin. The final thrombin enzyme effectively accommodates substrates at its serine protease active site and utilizes its mature exosites to regulate several coagulation related activities.

3429-Pos Board B157

Characterization of Proteases Derived from *Nephila Clavata*

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Spiders capture insects using a web net. The fact that they eat them without chewing suggests that spiders possess highly efficient digestive enzymes. Preliminary experiments indicated that a spider protease is able to digest synthetic spider dragline amyloid fibers. Thus, the spider protease has the potential ability to digest amyloid fibrils including pathogenic β-amyloid, such as amyloid fibrils, which are responsible for Alzheimer's disease. Therefore, we extracted and characterized the enzymes derived from *Nephila Clavata*.

Spider saliva including proteolytic enzymes was prepared from *Nephila Clavata* by electrical stimulation. The extracts were applied to SDS-PAGE and the enzymatic activity of spider proteases was estimated by a casein protease assay. Two protein bands, showing protease activity, were predominantly observed on the assay and their molecular weights were estimated as approximately 21.9 and 19.5 kDa, based on the SDS-PAGE analysis. In order to characterize the enzymes, an inhibition assay for a synthetic peptide substrate was performed using several types of inhibitors, such as PMSF and EDTA. The results suggested that the spider protease can be categorized as a metal-dependent carboxypeptidease although the inhibitors were not able to completely depress the protease activity of the enzyme. The results will be discussed in this paper.

3430-Pos Board B158

Structural and Computational Studies of the Staphylococcus Aureus Sortase B-Substrate Complex Provide New Insight into the Mechanism of Sortase Transpeptidases

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Sortase cysteine transpeptidases covalently attach proteins to the bacterial cell wall or assemble fiber-like pili that promote bacterial adhesion. Members of this enzyme superfamily are widely distributed in Gram-positive bacteria which frequently utilize multiple sortases to elaborate their peptidoglycan. Sortases catalyze transpeptidation using a conserved active site His-Cys-Arg triad that joins a sorting signal located at the C-terminus of their protein substrate to an amino nucleophile located on the cell surface. In order to understand the molecular basis of substrate recognition, we solved a crystal structure of the Staphylococcus aureus Sortase B enzyme (SrtB) in a covalent complex with an analog of its NPQTN sorting signal substrate. The results of computational modeling, molecular dynamics (MD) simulations, and targeted amino acid mutagenesis indicate that the backbone amide of Glu224 and the side chain of Arg233 form an oxyanion hole in SrtB which stabilizes high-energy catalytic intermediates. Surprisingly, a highly conserved threonine residue within the bound sorting signal substrate facilitates construction of the oxyanion hole by stabilizing the position of the active site arginine residue via hydrogen bonding. MD simulations and primary sequence conservation suggest that the sorting signal-stabilized oxyanion hole is a universal feature of enzymes within the sortase superfamily.

3431-Pos Board B159

The Role of Conformational Collapse in Enzymic Catalysis

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Lactate dehydrogenase catalyzes the inter-conversion between pyruvate and lactate, using NAD as cofactor. At the chemical step, hydride transfer from NADH to pyruvate C2=O carbon and proton transfer from protonated histidine to C2=O oxygen occur simultaneously on the ca. 20 fs time scale. Here, we investigate how (pig heart) lactate dehydrogenase (phLDH) guides the on-enzyme reaction pathway as the system goes from LDH•NADH•pyruvate to LDH•NAD+•lactate. Our previous studies on LDH•NADH•oxamate (a pyruvate analog) showed that this complex consists of two oxamate/NADH structures with different C2=O bond polarizations and different bending directions of the NADH ring. IR temperature-jump studies suggested these two structures do not interconvert directly but through a significantly less populated "encounter complex".

Here we report static FTIR and IR T-jump studies on LDH•NADH•pyruvate complex. This complex contains one major pyruvate structure in which pyruvate C2=O bond is significantly polarized. In addition, FTIR results can also identify at least three less populated pyruvate species with different C2=O bond polarizations. IR T-jump results suggest the pyruvate species with least C2=O bond polarization is likely the so called "encounter complex", which is the gateway leading to the formation of other pyruvate species with more polarized C2=O bond in the ground state. We argue that the bulk of the rate enhancement of an enzyme, using the chemistry catalyzed by LDH as a model system, is best viewed as arising from a quantitative consideration of the energy landscape specific to that found in solution compared to that found in the enzyme-substrate system. The chemical event in either medium is at the tail end of a stochastic search, probably largely Markovian, through an available phase space that, in the enzyme system, involves a restricted ensemble of more reactive conformational sub-states.

3432-Pos Board B160

The Role of Substrate Unbinding in Michaelis-Menten Enzymatic Reactions

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The Michaelis-Menten equation provides a hundred-year-old prediction by which any increase in the rate of substrate unbinding will decrease the rate of enzymatic turnover. Surprisingly, this prediction was never tested experimentally nor was it scrutinized using modern theoretical tools. Here we show that unbinding may also speed up enzymatic turnover - turning a spotlight to the fact that its actual role in enzymatic catalysis remains to be determined experimentally. Analytically constructing the unbinding phase-space, we identify four distinct categories of unbinding: inhibitory, excitatory, super-excitatory and restorative. A transition in which the effect of unbinding changes from inhibitory to excitatory as substrate concentrations increase, and an overlooked tradeoff between the speed and efficiency of enzymatic reactions, are naturally unveiled as a result. The theory presented herein motivates, and allows the interpretation of, groundbreaking experiments in which existing single-molecule manipulation techniques will be adapted for the purpose of measuring enzymatic turnover under a controlled variation of unbinding rates. As we hereby show, these experiments will not only shed first light on the role of unbinding, but will also allow isolated determination of the distribution of time required for the completion of a catalytic step embedded within a full enzymatic turnover cycle.

3433-Pos Board B161

Applying Osmotic Stress Reveals Two Modes of Enzyme Inhibition

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Enzymes realize their form and function in an aqueous environment filled with many other solutes. In addition to specific interactions, these co-solutes may also indirectly modify enzyme behavior through changes in osmotic pressure (i.e. the chemical potential of water). Using UV-visible spectrometry, we have measured the kinetics of bovine intestinal alkaline phosphatase catalyzed para-nitrophenol phosphate hydrolysis in the presence of various neutral solutes such as betaine, sucrose, triethylene glycol and polyethylene glycols. When analyzed using Lineweaver-Burk plots, the inhibition mechanism of these osmolytes appear analogous to "regular" small-molecule, mixed enzyme inhibitors (see figure). This suggests that these osmolytes can affect the two classic Michaelis-Menten "steps": substrate (S) binding to enzyme (E) (i.e. $E+S \leftrightarrow ES$), competitive inhibition, and the subsequent conversion of substrate to product (P) (i.e. $ES \rightarrow E+P$), uncompetitive inhibition. These observed effects could be interpreted as different degrees of osmolyte exclusion from regions on alkaline phosphatase critical to these steps. Furthermore, the amount and location of this exclusion would be sensitive to the steric and chemical differences between these solutes. These results highlight the importance taking into consideration the actually complex environment in which enzymes operate.